Changes in the Macromolecular Structures of Isolated Humic Substances Under Different Solution Chemical Conditions

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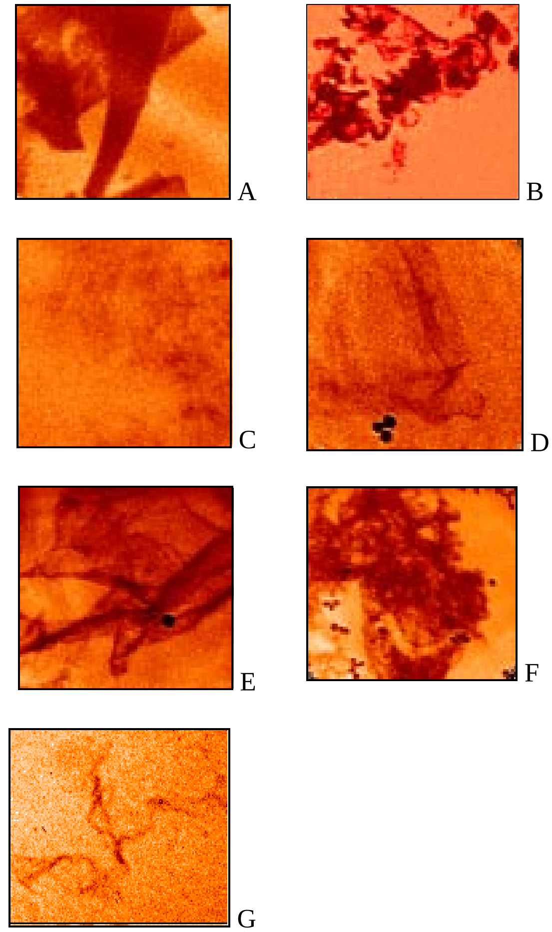
Humic substances (HS) are products of the biochemical transformations of plant and animal residues, and comprise a major fraction of the organic carbon of soil and aquatic systems[1]. Their reactivity in the environment is primarily dependent on their functional group chemistry and macromolecular structure (size, shape) induced by the composition of reacting media (solution chemistry, interacting solid matrix)[2,3]. However, direct (*in-situ*) information on the magnitude of these effects is yet to be documented. Since changes in HS macromolecular structures significantly affect the chemistry of organic coatings on soil mineral surfaces, and modify the retention of pollutants by soils and colloids, information on the magnitude of changes in HS macromolecular structures is essential for understanding the geochemical reactions mediated by natural organic molecules[4]. In this study, we examined the *in-situ* changes in the macromolecular structures of HS as a function of several chemical variables relevant to natural geochemical systems, using the X-ray microscopy facility (BL 6-1-2)[4]. Some of the results of this study are presented here.

EXPERIMENTAL DETAILS

Experiments were conducted on HS fractions (humic and fulvic acids) isolated from river water (Suwannee River, GA, U.S.A), and soil (mollic epipedon, IL, U.S.A) samples. These fulvic and humic acids are isolated at different pH by the International Humic Substance Society. The solution compositions tested were: pH (2-12), ionic strength (0.01-2 M), HS concentration (0.03-10 g C L^{-1}), counter ion composition (1 mM Cu^{2+}, Fe^{3+}), in the presence of soil minerals (goethite (α-FeOOH); calcite ($CaCO_3$); clay minerals (kaolinite and montmorillonite)). The macromolecular structures of aqueous HS under these chemical conditions were examined by collecting images at different photon energies.

RESULTS AND DISCUSSION

When examined with the X-ray microscope, the Suwannee River fulvic acid isolates formed aggregates of different shapes and sizes at HS concentrations greater than ~ 1.3 g L^{-1} (Fig. 1A). In dilute, acidic, high ionic strength NaCl solutions, HS predominantly formed globular aggregates and coils with a small radius of curvature (0.15 - 0.6 μm; Fig. 1B). As the fulvic acid concentration was increased, large sheet-like structures (2-8 μm) also formed. Visible coiling was uncommon and the HS dispersed completely into small aggregates (< 0.1 μm) in solutions of pH > 8.0 (Fig. 1C). Although addition of 1 M NaCl did not favor coiling under these alkaline conditions, concentrated HS solutions formed large globular aggregates bound together with thin films of HS. Additions of di- and trivalent cations to HS solutions promoted their precipitation at low carbon concentration, and displayed macromolecular structures different from those formed in the presence of monovalent ions. Additions of Ca^{2+} to fulvic acid at dilute concentration formed thin thread-, and net-like structures as compared to those formed in the presence of Na^+ (Fig. 1D, compare with Fig. 1B). Increases in Ca^{2+} and HS concentration caused these structures to grow larger and denser (Fig. 1 E-F). Coiling was also common in the concentrated Ca^{2+}-fulvic acid solutions (Fig. 1F). Humic solutions reacted with Cu^{2+} and Fe^{3+} also exhibit similar structures, but precipitated HS at a concentration less than those examined in the presence of Na^{+} and Ca^{2+} (Fig. 1G). The fluvial humic acids also showed the similar behavior, but the carbon concentration at which a particular structure was formed was smaller for the humic acids, and also globular and rod/lath-like structures were more common in the case of humic acids.



<u>Fig. 1</u>. Macromolecular structures of fluvial fulvic acid. **A**. redissolved solid fulvic acid in water, pH 4.0; **B**: pH 3.0, NaCl = 1.0 M; **C**: pH 9.0, NaCl = 0.5 M; **D**: [C] ~ 1.0 gL^{-1}, pH 4.0, $CaCl_2$ = 0.018 M; **E**: [C] ~ 1.3 gL^{-1}, pH 4.0, $CaCl_2$ = 0.07 M, **F**: [C] ~ 1.5 gL^{-1}, pH 4.0, $CaCl_2$ = 0.2 M; **G**: [C] ~ 0.1 gL^{-1}, Fe^{3+} = 1.0 mM.

In contrast with the fluvial humics, the low solubility of soil and peat HS promoted their precipitation at much lower HS concentration than the fluvial isolates (~ 0.25 g C L^{-1} for soil fulvic acid in acidic NaCl solutions, and at lower concentration for humic acids and in the presence of complexing cations). Although similar macromolecular structures were noticed for fluvial and soil HS below a pH of 8.0, the latter arranged preferentially in globular, and rod- or thread-like structures in dilute solutions (0.4 g C L^{-1}), and sheet-like structures (occasionally with open holes, which are uncommon for fluvial samples) in concentrated solutions (> 0.4 g C L^{-1}). In alkaline solutions, soil HS also dispersed at low HS concentration, and formed dense sheet-like structures in concentrated solutions.

The presence of mineral surfaces completely altered HS behavior. The size and structure of organo-mineral aggregates were dependent on the type (fulvic vs. humic acid) and concentration of HS, and the composition of minerals and solutions (pH and cation type and concentration). At low HS concentration (< aqueous saturation), sorption of HS was evident by the formation of thin coatings on the mineral surfaces that could only be resolved with surface-sensitive spectro-microscopy methods. In saturated soil HS solutions of pH 2-10, clay minerals, goethite, and fine-sized calcite formed organo-mineral aggregates with thick HS coatings. Kaolinite and montmorillonite exhibit the same behavior with the majority of their aggregates in the rage of 5-35 μm, and HS occurring as cement between the clay platelets. Although, alkaline soil humic acid solutions (pH > 8.0) showed the same behavior as fulvic acids, humic acids formed less dense aggregates with clays than the fulvic acids in the pH range of 2-7. Fine-grained calcite crystals exhibited less dense aggregates than those of clays, and sorbed more HS than the coarse calcite crystals below a pH of 8.0. As the HS concentration was increased well above its aqueous saturation, HS formed thick coatings around minerals, irrespective of their composition. All of the mineral samples, excepting those examined in dilute HS solutions, also exhibit separate non-mineralic-HS aggregates in globular and sheet-like forms. Although fluvial HS showed similar behavior with mineral matrices, detectable organo-mineral aggregates formed at high HS concentration (> 1.5 g C L^{-1}) only.

In summary, these results indicate that the macromolecular structures of HS are highly sensitive to different solution chemical conditions. The commonly held notion that HS form coils at low pH and high ionic strength, and elongated structures in alkaline solutions as the primary structures is too simplistic. However, the following generalizations can be made from this study: 1) HS were found to form globular and thread/net-like structures in dilute HS solutions, and coiled and sheet-like structures in concentrated HS solutions. Dilute alkaline HS solutions always exhibited small aggregates (< 100 nm) without any discernible structures. Solution pH significantly affected the HS macromolecular structures at low HS concentration, but played a minor role in the case of concentrated HS solutions. Humic and fulvic acid fractions of HS behaved similarly, but the chemical conditions under which transitions in their macromolecular structures occurred were different (more pronounced in the case of soil HS). In addition, humic acids formed globular and lath-shaped structures predominantly under acidic conditions. Although globular, and net-, and sheet-like structures have been reported earlier for dried soil HS on substrate surfaces (e.g. mica, sample mounting stubs for electron microscopes)[5], a correlation between their solution chemistry and HS structures could not be established. This may be because, chemical changes associated with sample drying (e.g. changes in HS concentration, pH), and sample substrate chemistry can modify the original HS macromolecular structures. 2) The mineral oxides dramatically alter the HS macromolecular structures, which vary depending on the chemistry and grain size of minerals. The chemical differences between fulvic and humic acids (aromaticity, aqueous solubility), notably in the case of soil HS, have caused different interactions with minerals. While soil humic acids predominantly formed structures without mineral oxides at pH < 7.0, soil fulvic acids formed organo-mineral aggregates with HS as cements between the minerals in the pH range of 2-10. 3) The chemical conditions under which soil and fluvial HS assume a particular configuration and interact with minerals are different. Soil HS were found to form globular, thread- and net-like structures in dilute HS solutions, and sheet-like structures in concentrated solutions at pH < 8.0. This behavior is more pronounced in the case of soil HS than the fluvial HS. The macromolecular structures of organo-mineral complexes in soils and those of isolates were similar, which suggests that the results obtained from the latter can be applied to understand the behavior of organic molecules in nature.

The results presented here directly document, for the first time, the variation in aqueous HS macromolecular structures as a function of solution chemical conditions and HS origin. The changes in HS macromolecular structures associated with solution chemical conditions modify the exposed surface area and the functional group chemistry of HS, which may further affect their biotransformation and contaminant sorption[6]. As this study shows, HS form dense structures with low surface area to volume ratio in acidic concentrated electrolyte solutions when compared to high pH solutions. This can significantly inhibit the accessibility of microorganisms into the micropores of dense HS aggregates, and thus prevents the oxidation of organic matter (and also other redox reactions of HS-associated contaminants), and facilitates the stabilization of organic carbon by soils. Differences in the macromolecular structures (e.g. size and C content) of mineral-complexed HS affect the properties of organo-mineral aggregates, and controls the type of organic carbon retained by different soil minerals. A description of the macromolecular structures of HS together with the associated chemical conditions will help the modeling and understanding the pollutant-particle interactions, and carbon cycling in soils.

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